Summary and Conclusion

The rate of global warming is expected to continue increasing if no mitigation efforts take place to reduce the carbon intensity of the world economy and the consequent emission of greenhouse gases (Raupach et al., 2007). Agricultural production and thus global food security, is directly affected by global warming (Fischer et al., 2005; Schmidhuber and Tubiello, 2007; Ainsworth and Ort, 2007). Temperature controls the rate of plant metabolic processes that ultimately influence the production of biomass fruits and grains (Hay and Walker, 1989). By 2080, most cropping areas in the world are likely to be exposed to record average air temperatures (Battisti and Naylor, 2009). High average “seasonal” temperatures can increase the risk of drought, limit photosynthesis rates and reduce light inception by accelerating phonological development (Tubiello et al., 2007). Previous global food assessments have shown that these negative effects are particularly exacerbated in tropical regions (IPCC, 2007a; Fischer et al., 2005). Heat stress damage is particularly severe when high temperatures occur concomitantly with critical crop developmental stages, particularly the reproductive period. Because of this, the fourth Assessment Report of the Intergovernmental Panel on Climate Change (IPCC) has acknowledged the heat stress as an important threat to global food supply (IPCC, 2007).

Elevated temperature a consequences of global climate temperature also has an adverse effect on crop productivity (Drigo et al., 2008). Heat stress negatively influences photosynthetic rate, plant water relations, flowering and fruit set in both tropical and temperature crops (Aborol and Ingram, 1996). The activation of genes responsive to heat stress is mediated by heat stress transcription factors (HsFs). Plant HsFs have a highly complex gene compromising of more than 20 members and the existence of Hs induced HsF genes are thought to modulate transcription during long term Hs response (Baniwal et al., 2004; Nover et al., 2001). Breeding for heat tolerant cultivars or development of transgenics is time consuming and not cost effective (Vanja et al., 2007. Therefore microbes may become useful in this regard. Earlier reports have suggested the usefulness of thermotolerant microbes in providing heat stress tolerance to plants Ali et al., 2009, Redman et al., 2002; Mclellan et al., 2007). Apart from thermotolerant bacteria reported, Mclellan et al., (2007) showed that the rhizosphere fungus Paraphrase sphaeria also enhanced
thermotolerance to *Arabidopsis thaliana* through induction of HSP 101 and HSp 70 proteins. These thermotolerant microbes may provide heat stress tolerance to plants through induction of biosynthesis of high molecular weight proteins in leaves, reduced membrane injury and increase the contents of cellular metabolites such as proline, chlorophyll, sugars, amino acids and proteins. Heat stress at reproductive stage is increasingly becoming a serious constraint to chickpea production because of expected increase in temperatures due to climate change (Summerfield *et al.*, 19810). To combat the problem, either transgenics or hybrid varieties are used but such stress tolerant varieties of all crops are presently not available while those that are available are very expensive bargain. Thermotolerant plant growth promoting microbes (PGPM) as an alternative has gained importance in recent years (Saloheimo and Pakula, 2012).

Filamentous fungi have become a boon for the biotechnology industry over the past decade, the main reasons being their ability to secrete large amounts of proteins (Salohrimo and Pakula, 2012) and the biocontrol capabilities of some species against nematodes (Gunasekera *et al.*, 2000), insects (Samson *et al.*, 1999) and phytopathogens (Papavizas, 1985; Chet, 1987). Mycoparasitic properties enable *T. harzianum* to protect a variety of plant crops from attack by a range of pathogenic species of fungi, (Hermosa *et al.*, 2012; Gunsekera *et al.*, 2000; Kullnig *et al.*, 2000), providing an environmentally benign alternative to chemical fungicides. Evidence shows that *T. harzianum* secretes a range of cell wall degrading enzymes (CWDEs) that break down the cell wall of phytopathogenic fungi, leading to death (Saloheimo and Pakula, 2012). Antibiotics are also secreted from *T. harzianum* during attack on phytopathogenic fungi (Schirmbock *et al.*, 1994; Lorito *et al.*, 1996).

**Specific objectives of the present study were as follow:**

- To isolate *Trichoderma* spp. from different heat stressed agro-ecosystems in India
- To screen and select the thermotolerant strain of *Trichoderma* spp.
- To study the comparative proteomic profiling of selected *Trichoderma* spp. under stressed and ambient conditions through 2D gel electrophoresis
- To study the effect of *Trichoderma* mediated reprogramming of oxidative stress markers and defense network in tomato to enhance thermal tolerance.
- To study the Root-*Trichoderma* interaction under heat stressed and ambient conditions.
Important findings of the present study are as follow:

- One hundred and fifty isolates of *Trichoderma* spp. was isolated from two hundred and seventy nine soil samples from 16 thermal stressed sites across 4 states in India. Colony growths of the 150 fungal isolates on PDA plates showed very different patterns at 15–45 °C. Of those, only seven isolates showed growth at 30 °C. Only two isolates viz. BHU3 and BHU4 were capable of growing from 15–45 °C. Conidial tolerance to the thermal stress of 47 °C differed greatly among the 7 fungal isolates assayed. BHU3 showed highest survival ratio when continuously stressed at 47 °C for five days closely followed by BHU4 which survived in the same conditions for four days. While BHU1, 5, 6, and 7 could not survive the heat stress after 75, 90 and 60 min, respectively.

- Molecular identification was performed by sequencing the 600–700 bp PCR product of the Internal Transcribed Spacer (ITS) region of 18S rDNA of the isolates. Out of the seven thermotolerant *Trichoderma* isolates, two best performing isolates (BHU3 and BHU4) were selected from previous experiments and were found to be *T. koningii* and *T. longibrachiatum*, respectively.

- *T. koningii* strain BHU3 showed the maximum inhibition percentage of mycelial growth against *Colletotrichum capsici* (96.14 %) followed by *Macrophomina phaseolina* and *Bipolaris sorokiniana* (83.33 %), *Fusarium oxysporum* (80.3 %), *Phoma* sp. (76.3 %) while in case of *Sclerotium rolfsii, Sclerotinia sclerotiorum* and *Rhizoctonia bataticola* the inhibition percentages were 72 %, 65 % and 55%, respectively.

- Experiments qualitatively demonstrated that *T. koningii* strain BHU3 had retained atleast 75% of cellulolytic activity, 80% pectinolytic activity, 40% siderophore production activity and 55% chitinolytic activity under prolonged heat stress. Thus, representing very high biocontrol potential of this strain to perform under thermal stress. *T. koningii* strain BHU3 showed a production of 46.71 ± 2.12 μg/ ml IAA and 18.60 ± 1.70 μg/ ml IAA under ambient conditions and heat stress, respectively. Thus demonstrating 40% retention of IAA production ability under heat stress of 45 °C. 298.75 ± 10.75 μg/ ml Pi and 175.80 ± 8.63 μg/ ml inorganic phosphate was solubilized under ambient and heat stress conditions, respectively. Thus demonstrating 59% retention of phosphate solubilization ability under heat stress of 45 °C.
• Development of easily reproducible methodology for the separation of as many proteins as possible, using 2-DE technology, from a thermotolerant strain of *T. koningii* BHU3 leading to production of an initial protein reference map for investigating the comparative proteomic profile under heat stress.

• Effect of *Trichoderma* mediated reprogramming of oxidative stress markers and defense network in tomato to enhance thermal tolerance was also investigated. Four treatments were given to the plants. T₁- Seeds without *Trichoderma* treatment; T₂- Seed without *Trichoderma* treatment + Heat Stress; T₃- Seed with *Trichoderma* treatment and T₄- Seed with *Trichoderma* treatment + Heat Stress. PO activity varied from 5.06 ± 0.22 to 2.178 ± 0.14 min⁻¹ g⁻¹ FW, with a maximum activity in leaf extracts of T₂. In T₃ the activity of PO was 4.077 ± 0.22 and in T₄ it was observed to be 3.87 ±0.13. On comparing the obtained data with heat stressed and untreated control a significantly reduced activity of PO was observed in treated and stressed condition.

• PPO activity was more in untreated leaf extracts as compared to the *Trichoderma* treated leaf extracts. PPO activity varied from 71.46 ± 0.47 to 22.72 ± 0.32 min⁻¹ g⁻¹ FW, with a maximum activity observed in T₂, which was significantly higher than the treated leaf extracts i.e. T₄. Maximum content of starch was observed in case of T₁ followed by T₃. A significant increase in the protein content was seen in case of T₄, a lower level of protein was seen in case of tomato seed inoculated with T₃. A comparatively higher level of protein was seen in case of control as compared to T₂.

• The effect of *Trichoderma* inoculation and heat stress on peroxidase activity was determined on leaf tissues. The peroxidase level was found highest in T₂, followed by tomato seeds treated with T₃. A comparatively lower level of H₂O₂ was observed in case of tomato seeds treated with T₄.

• Host plant root –*Trichoderma* interaction under heat stressed and ambient conditions was also studied. Germination percentage of seeds treated with *T. konongii* strain BHU3 was calculated and average germination percentage of 97.23% ± was observed in treated set in comparision to 93.5% ± 3% of the untreated control. Radicle of seedling treated with *T. koningii* strain BHU3 showed average growth of 4.36 cm ± 0.15 cm while the untreated control showed an average radical growth of 3.70 cm ± 0.22 cm. Conferring that treatment with *T. koningii* strain BHU3 effectively increasec the germination
percentage by 3.73%, radical length by 29.45%, plumule length by 30.5% and fresh weight of rice seedlings by 35.6% in comparison to the untreated control.

- It is found from the quantitative estimation data of total free phenol content that the plants treated with *Trichoderma koningii* strain BHU3 exhibit significantly higher free phenol content as compared to the untreated and unstressed control. Rice seedlings were grouped into four sets including, the untreated and unstressed control (T1), heat stressed untreated seedlings (T2), *Trichoderma* treated, unstressed seedlings (T3) and *Trichoderma* treated and heat stressed seedlings (T4). After three weeks of growth all the sample had predominantly shikimic acid (SA) and gallic acid (GA in the root exudates).

Thus, overall it can be concluded that these observations enhance potential interest in the use of *T. koningii* as a beneficial microorganism for lowering down the adverse effect of higher temperature upto a tolerable level and increasing the crop yield. Though, a deeper investigation on the mechanism of *T. koningii* strain BHU3 in thermal stress mitigation in crops would help in developing strategies for improving the efficacy of *Trichoderma* especially in field conditions.